Lung Vascular Effects of Lipid Infusion in Awake Lambs

WILLIAM G. TEAGUE, JR., J. USHA RAJ, DAVID BRAUN, MICHEL E. BERNER,
RONALD I. CLYMAN, AND RICHARD D. BLAND

*Cardiovascular Research Institute and Department of Pediatrics University of California, San Francisco,
San Francisco, California 94143*

ABSTRACT. Numerous reports have shown that intravascular lipid infusion may cause pulmonary dysfunction in a variety of species, including humans. To determine the effects of parenteral lipid on neonatal pulmonary hemodynamics, lung fluid filtration, and respiratory gas exchange, we measured pulmonary arterial and left atrial pressures, cardiac output, lung lymph flow, lymph and plasma protein concentrations, and partial pressures of oxygen and carbon dioxide in arterial blood of 11 awake chronically catheterized lambs that received a 2–3 h control infusion of glucose-saline solution followed by Intralipid at a dose of 67.5, 125, or 250 (mg/h)/kg body weight for 6 h. Intralipid caused an acute dose-dependent increase in pulmonary arterial pressure, with no significant change in cardiac output or left atrial pressure. The pulmonary hypertension, which lasted for at least 2 h, was accompanied by a >50% increase in lung lymph flow and a significant decrease in lymph protein concentration relative to plasma protein concentration. Pulmonary artery pressure gradually decreased to control values during the final 2 h of lipid infusion, but lymph flow remained 35% above control and lymph protein concentration remained low. Lipid infusion also was associated with a modest decrease in PaO_2 . Both arterial and venous administration of lipid gave similar results. In separate studies, lipid infusion caused a significant increase in plasma concentrations of thromboxane B_2 , the stable metabolite of thromboxane A_2 , without affecting plasma concentrations of 6-keto prostaglandin $\text{F}_{1\alpha}$, the stable metabolite of prostacyclin. Pretreatment with imidazole, which attenuated the lipid-induced increase in thromboxane B_2 concentration, completely blocked the pulmonary hemodynamic response to lipid. Moreover, in the presence of imidazole there was no significant change in any of the other measured variables during lipid infusion. These results suggest that 1) lipid infusion causes a sustained increase in pulmonary microvascular pressure, most likely from constriction of lung vessels distal to sites of fluid exchange; and 2) thromboxane A_2 is at least partly responsible for these changes. (*Pediatr Res* 22:714–719, 1987)

Abbreviations

Tx, thromboxane
6-keto-PGF $_{1\alpha}$, 6-keto prostaglandin $\text{F}_{1\alpha}$

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Correspondence to Richard D. Bland, M.D., Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA 94143.

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PaO_2 , partial pressure of O_2 in arterial blood
 PaCO_2 , partial pressure of CO_2 in arterial blood

Infants and children with feeding intolerance often receive intravenous infusion of lipid as a source of essential fatty acids and calories. This nutritional support may promote healing and hasten growth, but it also may impair lung function, with an associated reduction in arterial oxygen tension (1) and pulmonary diffusing capacity (2, 3), and an increase in lung vascular resistance (4, 5).

The mechanisms by which lipid infusion may interfere with respiratory gas exchange and cause pulmonary vasoconstriction in humans remains unclear. One possible explanation is that emulsified fat lodges in lung capillaries and thereby blocks blood flow. In support of this theory, several groups of investigators (6–8) have noted the presence of fat in the pulmonary microcirculation of infants who have died after treatment with intravenous lipid. However, similar fat deposits occur in the lung vasculature of infants who have not received parenteral lipid (9). This observation raises doubt about the possible link between lipid infusion and pulmonary emboli.

An alternative explanation for the lung dysfunction that develops during lipid infusion is that release of vasoactive substances may disrupt the normal relationship between pulmonary perfusion and ventilation. At least two previous reports have provided evidence in support of such a theory. McKeen *et al.* (10) found that pretreatment of sheep with indomethacin, a prostaglandin synthesis inhibitor, prevented the increase in pulmonary arterial pressure and lung lymph flow associated with intravenous infusion of emulsified fat. In a subsequent study performed with isolated adult rabbit lungs, Gurtner *et al.* (11) showed that addition of lipid emulsion to the perfusate caused vasoconstriction and significant increases in the concentrations of two cyclooxygenase-derived products of arachidonic acid metabolism, TxB_2 and 6-keto-PGF $_{1\alpha}$, in pulmonary venous outflow liquid.

The purpose of this work was to study the effects of lipid infusion on pulmonary vascular pressures and lung fluid filtration in unanesthetized newborn lambs. We found that lipid, delivered either through a systemic vein or artery, caused increases in pulmonary artery pressure and lung lymph flow which were associated with increases in plasma concentrations of TxB_2 . Pretreatment of the lambs with imidazole, an inhibitor of thromboxane synthesis, prevented both the chemical and physiological changes, suggesting that specific products of arachidonic acid metabolism, rather than embolic phenomena, probably account for the pulmonary abnormalities that develop during intravenous lipid therapy.

METHODS

Preparation for experiments. We surgically prepared 11 newborn lambs for measurement of systemic and pulmonary vascular pressures, cardiac output, and lung lymph flow, which under steady state conditions reflects net transvascular filtration of fluid in the lungs (12, 13). By methods previously described (14, 15), each lamb had two thoracotomies under halothane and nitrous oxide anesthesia delivered through a piston-type respirator. Before and after surgery the lambs remained with their ewes for feeding and warmth.

During the first thoracotomy, performed when the lambs were less than 3 days old, we inserted polyvinyl catheters directly into the pulmonary artery and left atrium, and we also placed a 3 Fr thermistor (Gould Statham, model SP5003, Gould, Inc.) in the pulmonary artery. During the same procedure, we threaded catheters into the abdominal aorta and inferior vena cava through a leg incision. After recovery from the first surgery, usually 4–5 days later, the lambs underwent a right thoracotomy, in which we resected systemic contributions to the caudal mediastinal lymph node and inserted a heparin-coated polyvinyl catheter (inner diameter 0.35 mm, outer diameter 0.89 mm) into the efferent duct of this node, which drains about two-thirds of total lung lymph in lambs (16). We tunneled the catheter beneath the pleura and through the chest wall, and secured it to the skin with a suture. We also placed a 4 × 2 cm silicone (Silastic, Dow Corning) balloon catheter in the pleural space for subsequent monitoring of respirations and pleural pressure. Vinyl pouches, sewn to the sides of the lambs, protected the catheters from being chewed by the ewes. The lambs had at least 3 days to recover from surgery before experiments began.

Description of experiments. The average age of the lambs at the time of the experiments was 23 days (range 11–27 days) and their average weight was 7.8 kg (range 4.6–11.2 kg). During studies the lambs were awake and rested in a prone position on a canvas sling beneath a radiant warmer that kept body temperature constant. We intermittently flushed all vascular catheters with 1 ml of isotonic saline containing 10 U of heparin/ml to prevent clotting. We continuously measured pressures in the pulmonary artery, left atrium, aorta, vena cava, and pleural space with calibrated transducers (Gould Statham P23DC and 131TC, Gould, Inc.) connected to a 6-channel amplifier-recorder (Grass Instruments). Every 30 min we averaged these measurements and recorded their mean values. Zero reference level for measurement of vascular pressures was a line drawn on the lambs' skin at the level of the left atrium at the time of surgery. We measured cardiac output by thermal indicator dilution (17) every 2–4 h and recorded the mean value for three consecutive measurements. We collected lung lymph every 30 min in heparinized test tubes, with the distal end of the lymph catheter situated at the level of the left atrium, and we measured the volume of each sample of lymph to the nearest 0.01 ml. We collected samples of arterial blood hourly for measurement of packed cell volume (hematocrit), pH, blood gas tensions, and plasma protein concentration.

Studies of lipid infusion and lung fluid filtration. We did 17 experiments with 11 lambs in which we gave a continuous intravenous or intraarterial infusion of lipid (Intralipid, Cutter Laboratories) as we measured vascular pressures, cardiac output, lung lymph flow, and protein concentrations in lymph and plasma (the composition of Intralipid: soybean oil, 10%; egg yolk phospholipids, 1.2%; and glycerin, 2.25%). The major fatty acid components are: linoleic, 50%; and oleic, 26%). In the control period, the lambs received a continuous infusion of 5% glucose and 75 mM NaCl administered by infusion pump (Harvard Instruments) for 2–4 h at a rate of 15–25 ml/h. We then switched the infusion from the crystalloid solution to the lipid emulsion, which we infused for 6 h, sufficient for all variables to reach a steady state. We designated the first 2 h as the acute phase and

the last 2 h as the steady state phase of the experiments. To see if the site of infusion might influence the outcome, we infused lipid intravenously in six lambs and intraarterially in five lambs.

We used three doses of lipid within a clinically relevant range: in four experiments we infused lipid at a rate of 67.5 (mg/h)/kg body weight; in seven experiments we gave 125 (mg/h)/kg; and in six experiments we used a dose of 250 (mg/h)/kg. To keep the infusion volumes similar, we adjusted the concentration of lipid rather than the rate of administration of fluid for each series of experiments.

Studies of lipid infusion and arachidonic acid metabolites. In eight separate lambs, which did not have lymph cannulae, we measured pulmonary vascular pressures, cardiac output, and arterial plasma concentrations of TxB_2 and 6-keto- $\text{PGF}_{1\alpha}$. The average age of these lambs was 13 days (range 6–18 days) and their average weight at the time of studies was 10.3 kg (range 6.9–15.0 kg). After a 2-h control period in which we infused isotonic saline at a rate of 2.5 ml/kg hourly, we infused lipid emulsion at a rate of 250 (mg/h)/kg for 1 h. We obtained arterial blood samples for measurement of TxB_2 and 6-keto- $\text{PGF}_{1\alpha}$ during the control period and 30 and 60 min after starting the lipid infusion.

Four of these lambs (average age 16 days, range 10–18 days; average weight 8.7 kg, range 7–11 kg) had paired experiments in the presence and absence of imidazole, an inhibitor of thromboxane synthetase. To perform the inhibition studies, we infused imidazole intravenously at 50 mg/kg for 30 min before we started the lipid, after which we infused imidazole continuously at a dose of 30 (mg/h)/kg in conjunction with the lipid infusion, given initially at a dose of 100 (mg/h)/kg for 1 h followed by 250 (mg/h)/kg for 1 h. We alternated the sequence of the studies with and without imidazole treatment: two lambs had lipid alone first and two received the combination of imidazole and lipid for their initial study.

Studies of thromboxane synthetase inhibition and lung fluid filtration during lipid infusion. In five of the 11 lambs that had lymph catheters, we intravenously infused imidazole, 50 mg/kg, for 1 h, after which we continued the imidazole infusion at a dose of 30 (mg/h)/kg for 4–6 h while we simultaneously infused lipid at a dose of 250 (mg/h)/kg. We prepared the imidazole (Sigma Chemical Co.) solution on the day of each experiment by mixing 50 mg/ml of isotonic saline. During these studies, we measured vascular pressures, cardiac output, and lung lymph flow, just as we did in the lipid infusion studies without imidazole.

Biochemical measurements. During experiments we measured protein concentrations in lymph and plasma with a refractometer (American Optical Co.) and for greater precision we subsequently repeated the protein measurements by the biuret method (18). Cellulose acetate electrophoresis (Microzone 110, Beckman Instruments) was used to measure subfractions of albumin and globulin in the samples of lymph and plasma that we saved from the experiments.

To measure the concentrations of TxB_2 and 6-keto- $\text{PGF}_{1\alpha}$ in plasma, we collected 5 ml of arterial blood in cold, heparinized syringes and transferred the blood to cold test tubes that contained indomethacin, 2 $\mu\text{g}/\text{ml}$ blood. After centrifugation of the blood at $2000 \times g$ at a temperature 4°C for 20 min, we removed the supernatant plasma which we froze at -70°C for subsequent extraction and column chromatography. We mixed 2 ml of plasma with 3000 dpm of $^3\text{H-TxB}_2$ or $^3\text{H-6-keto-PGF}_{1\alpha}$ (110–130 Ci/nmol, New England Nuclear) for calculation of recovery. The samples were acidified to pH 4.0 with citric acid, after which they were extracted with a cyclohexane:ethyl acetate mixture for TxB_2 or ethyl acetate alone for 6-keto- $\text{PGF}_{1\alpha}$. The residues were stored at -20°C .

We measured TxB_2 or 6-keto- $\text{PGF}_{1\alpha}$ by radioimmunoassay after their separation by silicic acid chromatography (19, 20). For each concentration point of the standard curve for TxB_2 or

6-keto-PGF_{1α}, we carried a 2-ml sample of lamb plasma, preincubated with activated charcoal to remove TxB₂ and 6-keto-PGF_{1α}, through the purification process used for the 1-ml experimental plasma samples. Appropriate TxB₂ or 6-keto-PGF_{1α} standards were added to each of these evaporated residues. This procedure yielded reproducible standard curves with linear titration from 1–100 pg TxB₂ and 10–500 pg 6-keto-PGF_{1α}. Antisera to TxB₂ or 6-keto-PGF_{1α} were obtained from Seragen Inc. Concentrations are expressed as free acid.

Statistical analysis. Results in the text and tables are expressed as the mean $\pm$ SD. We used 2-way analysis of variance (21) to compare measurements made in the control period with measurements made in the two experimental periods (acute and steady state). If analysis of variance showed a significant difference, $p < 0.05$, we then used the Newman-Keuls test to compare measurements obtained during the control and experimental periods. We used Student's *t* test for paired samples to compare results for the five lambs that had lymph experiments conducted with and without imidazole. For all statistical analyses, we regarded as significant those differences that were supported by $p < 0.05$.

RESULTS

Studies of lipid infusion and lung fluid filtration. Lipid infusion at a rate of 125 (mg/h)/kg and 250 (mg/h)/kg caused an abrupt increase in pulmonary arterial pressure, with no significant change in left atrial pressure (Table 1, Fig. 1). Lung lymph flow increased and the concentration of protein in lymph relative to that in plasma decreased as pulmonary hypertension developed during the acute phase of these studies. In the steady state period, 4–6 h after the start of the lipid infusion, pulmonary arterial pressure decreased to a value at or just above control; lymph flow also decreased but remained 35% greater than it was during the control period. The concentration of protein in lymph relative to that in plasma remained less than the control value during the steady state period.

Lipid infusion had a dose-dependent effect on both pulmonary arterial pressure and lung lymph flow during the acute, but not during the steady state, phase of these experiments (Fig. 1, Table 1). At the lowest dose, 67.5 (mg/h)/kg, lipid had no significant effect on any of these measurements. At the highest dose, 250

Table 1. Dose-related effects of intravascular lipid infusion on pulmonary vascular pressures, blood flow, lymph flow, and lymph protein concentration relative to plasma protein concentration in lambs (mean $\pm$ 1 SD)

Infusion dose [(mg/h)/kg]	n	Experimental period	Vascular pressure		Cardiac output (liter/min)	Lung lymph flow (ml/h)	Lymph protein concentration
			Pulmonary artery (torr)	Left atrium (torr)			Plasma protein concentration
67.5	4	Control	16 $\pm$ 1	1 $\pm$ 1	2.2 $\pm$ 0.1	2.02 $\pm$ 0.22	0.65 $\pm$ 0.06
		Acute	18 $\pm$ 3	0 $\pm$ 1	2.3 $\pm$ 0.2	2.96 $\pm$ 0.30	0.64 $\pm$ 0.07
		Steady state	18 $\pm$ 2	1 $\pm$ 1	2.3 $\pm$ 0.2	2.68 $\pm$ 0.26	0.64 $\pm$ 0.06
125	7	Control	15 $\pm$ 1	0 $\pm$ 1	2.2 $\pm$ 0.4	2.44 $\pm$ 0.32	0.61 $\pm$ 0.03
		Acute	21 $\pm$ 4*	0 $\pm$ 1	2.1 $\pm$ 0.5	4.00 $\pm$ 0.72*	0.53 $\pm$ 0.02*
		Steady state	17 $\pm$ 2	0 $\pm$ 1	2.1 $\pm$ 0.5	3.24 $\pm$ 0.48*	0.54 $\pm$ 0.03*
250	6	Control	16 $\pm$ 4	1 $\pm$ 1	2.5 $\pm$ 0.5	2.48 $\pm$ 0.78	0.60 $\pm$ 0.04
		Acute	25 $\pm$ 6*	1 $\pm$ 2	2.4 $\pm$ 0.4	4.44 $\pm$ 0.96*	0.46 $\pm$ 0.04*
		Steady state	18 $\pm$ 3	1 $\pm$ 1	2.5 $\pm$ 0.5	3.40 $\pm$ 0.64*	0.51 $\pm$ 0.04*

* Significant difference, $p < 0.05$, compared to control values.

Fig. 1. Summary results for experiments in which lambs received intravascular lipid at a rate of 125 (mg/h)/kg (seven lambs) or 250 (mg/h)/kg (six lambs) for 6 h. Lines represent mean values and stippled areas represent $\pm$ 1 SD. The first 2 h of lipid infusion is designated "acute phase" and the last 2 h "steady state phase."

(mg/h)/kg, the acute increases in pulmonary arterial pressure and lung lymph flow were greater than the increases that occurred when the lambs received 125 (mg/h)/kg. With respect to the site of infusion, both arterial and venous administration of lipid produced the same increases in pulmonary arterial pressure and lung lymph flow. Neither arterial nor venous administration of lipid had a significant effect on cardiac output or systemic vascular pressures.

Lipid infusion also led to a consistent but modest decrease in arterial oxygen tension (Table 2). The reduction in PaO_2 was sustained during the entire 6 h of lipid infusion at a dose of 250 (mg/h)/kg, whereas the PaO_2 returned to the control value at the lower dose. Lipid infusion at all three doses had no significant effect on respiratory rate or pleural pressure (data not shown). PaCO_2 , or arterial pH.

Studies of lipid infusion and arachidonic acid metabolites. Lipid infusion was associated with an increase in plasma concentrations of TxB_2 (Table 3). Plasma concentrations of 6-keto- $\text{PGF}_{1\alpha}$ did not change significantly during lipid infusion. In each of four paired studies, with and without thromboxane synthetase inhibition, imidazole attenuated the increase in plasma concentration of TxB_2 during lipid infusion (Table 4).

Studies of thromboxane synthetase inhibition and lung fluid filtration. Pretreatment with imidazole completely blocked the acute increases in pulmonary arterial pressure and lung lymph flow caused by lipid infusion (Fig. 2). In the control period, imidazole alone had no effect on lung vascular pressures or lymph flow. When we switched the infusion to lipid, all measurements remained constant. Imidazole also inhibited the decrease in arterial oxygen tension associated with lipid infusion.

DISCUSSION

These results show that intravascular infusion of lipid in normal lambs causes a significant increase in plasma thromboxane concentration, accompanied by acute pulmonary hyperten-

sion and a sustained increase in pulmonary lymph flow, indicative of increased lung fluid filtration. The associated reduction in lymph protein concentration suggests that the increase in fluid filtration was the result of elevated pressure in the pulmonary microcirculation, rather than abnormal endothelial permeability. Figure 3 illustrates this point more clearly by comparing the relationship between lung lymph flow and lymph protein concentration relative to plasma protein concentration for two sets of experiments: in one series of studies, we infused lipid at a rate of 250 (mg/h)/kg body weight, and in the other series of studies (13) we increased lung microvascular pressure by injecting saline into a balloon catheter situated in the left atrium of seven healthy lambs. In both sets of experiments, the concentration of protein in lymph relative to that in plasma decreased as lymph flow increased. The magnitude of the changes was greater in the

Table 4. Plasma concentrations of TxB_2 and 6-keto $\text{PGF}_{1\alpha}$ in four lambs during intravenous lipid infusion in the absence and in the presence of imidazole, 50 mg/kg, followed by 30 (mg/h)/kg (mean $\pm$ 1 SD)*

Lipid dose [(mg/h)/kg]	Time† (min)	Imidazole	TxB_2 (pg/ml)	6-keto- $\text{PGF}_{1\alpha}$ (pg/ml)
Control		—	93 $\pm$ 40	22 $\pm$ 4
100	30	—	231 $\pm$ 164	34 $\pm$ 20
250	30	—	370 $\pm$ 411	39 $\pm$ 26
	60	—	356 $\pm$ 318	33 $\pm$ 9
Control		+	81 $\pm$ 45	33 $\pm$ 17
	30	+	130 $\pm$ 41	38 $\pm$ 6
	30	+	169 $\pm$ 106	60 $\pm$ 7
	60	+	111 $\pm$ 30	36 $\pm$ 13

* TxB_2 concentrations increased at both concentrations in all four experiments without imidazole; the increase was attenuated in the presence of imidazole.

† Time after start of lipid infusion at the given dose.

Table 2. Dose-related effects of intravascular lipid infusion on variables related to respiration and gas exchange in lambs (mean $\pm$ 1 SD)

Infusion dose [(mg/h)/kg]	n	Experimental period	PaO_2 (torr)	PaCO_2 (torr)	pH
67.5	4	Control	87 $\pm$ 9	39 $\pm$ 2	7.40 $\pm$ 0.02
		Acute	87 $\pm$ 2	40 $\pm$ 2	7.40 $\pm$ 0.02
		Steady state	87 $\pm$ 2	40 $\pm$ 2	7.40 $\pm$ 0.02
125	7	Control	84 $\pm$ 5	40 $\pm$ 4	7.40 $\pm$ 0.03
		Acute	78 $\pm$ 7*	39 $\pm$ 3	7.39 $\pm$ 0.02
		Steady state	82 $\pm$ 6	38 $\pm$ 4	7.39 $\pm$ 0.03
250	6	Control	88 $\pm$ 8	39 $\pm$ 3	7.41 $\pm$ 0.04
		Acute	78 $\pm$ 7*	40 $\pm$ 3	7.39 $\pm$ 0.03
		Steady state	74 $\pm$ 9*	40 $\pm$ 4	7.40 $\pm$ 0.02

* Significant difference, $p < 0.05$, compared to control values.

Table 3. Effects of lipid infusion at 250 (mg/h)/kg on pulmonary hemodynamic variables and on plasma concentrations of TxB_2 and 6-keto- $\text{PGF}_{1\alpha}$ in eight lambs (mean $\pm$ 1 SD)

	Vascular pressures		Cardiac output (liter/min)	Pulmonary vascular resistance [torr/(liter/min)]	Plasma TxB_2 (pg/ml)	Plasma 6-keto- $\text{PGF}_{1\alpha}$ (pg/ml)
	Pulmonary artery (torr)	Left atrium (torr)				
Control	16 $\pm$ 3	3 $\pm$ 1	3.0 $\pm$ 0.6	5.3 $\pm$ 1.6	107 $\pm$ 53	165 $\pm$ 148
Lipid infusion	26 $\pm$ 6*	3 $\pm$ 1	3.0 $\pm$ 0.7	9.3 $\pm$ 4.6*	296 $\pm$ 282† 254 $\pm$ 236‡	154 $\pm$ 158† 160 $\pm$ 142‡

* Significant difference from control, $p < 0.05$.

† Sample drawn 30 min after start of lipid infusion.

‡ Sample drawn 60 min after start of lipid infusion.

Fig. 2. Summary results of five studies in which lambs received imidazole, 50 mg/kg over a 1-h period, followed by 30 (mg/h)/kg for 5 h during concurrent intravascular infusion of lipid, 250 (mg/h)/kg. Lines represent mean values and stippled areas represent ± 1 SD.

Fig. 3. Relationship between lymph/plasma protein concentration and lung lymph flow for two sets of experiments in lambs. In one set, lymph flow was increased by saline-filling a balloon in the left atrium to raise lung microvascular pressure by an average of 10 torr, and in the other set of experiments lymph flow was increased by intravascular infusion of lipid at a rate of 250 (mg/h)/kg for 6 h. Results are mean ± 1 SD for seven pressure elevation studies (solid symbols) and six lipid infusion studies (open symbols).

pressure elevation studies because lung microvascular pressure increased by more than 10 torr, considerably greater than the pressure rise that occurred during lipid infusion. The point that we wish to emphasize is that the slopes of the two lines are virtually identical, indicating that protein sieving was similar in the two sets of experiments and that the probable source of the increased lymph flow during lipid infusion was increased filtration pressure.

The fact that lymph flow remained high and lymph protein concentration remained low during the steady state phase of the lipid infusion studies, when pulmonary arterial pressure was almost back to control values, suggests that lipid caused a persistent increase in lung vascular resistance distal to sites of fluid

exchange, possibly in pulmonary venules. Thus, the pulmonary hemodynamic effects of lipid infusion include an initial dose-dependent increase in pulmonary vascular resistance, associated with elevated plasma concentrations of thromboxane, followed by a persistent increase in fluid filtration pressure. It is likely that this pressure rise occurs in the venular and microvascular segments of the pulmonary circulation, with a secondary increase in fluid filtration into the lungs. All of these changes can be blocked by concurrent administration of imidazole, which attenuates the lipid-induced increase in the concentration of thromboxane in plasma.

We have no reason to believe that the lipid-induced increase in lymph flow was the result of altered lung vascular permeability to protein. The concentration of protein in lymph decreased during lipid infusion, whereas lymph protein concentration usually increases or remains constant when endothelial injury occurs (13, 22). It is also unlikely that the increase in lymph flow was the result of pulmonary fat embolism, as intraarterial infusion of lipid had the same effect as did intravenous infusion. Furthermore, McKenney *et al.* (10) previously showed that heparin administration to adult sheep at a dose sufficient to clear their plasma or triglycerides did not alter their pulmonary vascular and lymph flow response to lipid infusion.

The source of thromboxane during lipid infusion is unknown. It is possible that the lipid infusate itself contains free arachidonic acid, which has been shown to constrict pulmonary venules in isolated, perfused dog lungs (23). Exogenous arachidonic acid infused into newborn lambs causes all of the hemodynamic changes that we observed during lipid infusion, and it also increases plasma concentrations of TxB_2 without increasing 6-keto-PGF $_{1\alpha}$ (24). In addition, lipid infusion may stimulate endogenous tissue release of TxA_2 , with secondary pulmonary vasoconstriction.

In addition to their potent effect on pulmonary vascular smooth muscle, arachidonic acid metabolites of both the cyclooxygenase and lipoxygenase pathways may cause bronchoconstriction (25), with resultant mismatch of ventilation and perfusion. Inhibition of Tx synthetase either attenuates or prevents these changes from occurring in sheep with either complement-mediated or endotoxin-induced lung injury (26, 27). We did not measure lung mechanics in our lambs, but it is possible that a

change in airway resistance may have contributed to the decrease in arterial oxygenation associated with lipid infusion. Our results are consistent with the previous observation that cyclooxygenase inhibition lessened the effect of lipid infusion on arterial oxygenation in rabbits with lung injury from oleic acid (28). It is extremely unlikely that the modest increase in lung fluid filtration that occurred during only 6 h of lipid administration had any influence on respiratory gas exchange in our lambs.

Certain features of this work may have important clinical implications concerning lipid delivery in patients with lung disease. First, there appears to be a threshold dose below which lipid does not cause an appreciable increase in lung fluid filtration. Our data suggest that in healthy lambs, an infusion rate of 67.5 (mg/h)/kg body weight, or 1.6 (g/day)/kg, does not significantly increase either pulmonary arterial pressure or lung lymph flow. Second, because the acute pulmonary vascular response to lipid is considerably greater than the steady state response, it seems sensible that lipid should be given continuously, rather than intermittently, over a 24-h period in order to avoid the abrupt changes associated with initiation of infusions. Finally, it is possible that these potentially adverse effects of lipid infusion can be prevented by development of safe and effective methods to inhibit thromboxane synthesis either by pharmacological means or by altering the fatty acid composition of the infusate. If lipid infusion affects pulmonary vascular resistance, lung fluid filtration, and arterial oxygenation in human infants as it does in lambs, then suitable measures to inhibit these effects may help to prevent the development of pulmonary edema and respiratory dysfunction in babies that require parenteral nutrition with fat.

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